CLAIMS

1. A method of producing a template DNA used for protein synthesis comprising a step of

amplifying a linear double-stranded DNA by polymerase chain reaction (PCR), using a reaction solution comprising,

a first double-stranded DNA fragment comprising a sequence coding for a protein or a portion thereof,

a second double-stranded DNA fragment comprising a sequence overlapping with the 5' terminal region of the first DNA fragment,

a third double-stranded DNA fragment comprising a sequence overlapping with the 3' terminal region of the first DNA fragment,

a sense primer which anneals with the 5' terminal region of the second DNA fragment, and

an anti-sense primer which anneals with the 3' terminal region of the third DNA fragment,

wherein the second DNA fragment comprises a regulatory sequence for transcription and translation of a gene, and the concentrations of the second DNA fragment and the third DNA fragment in the reaction solution each range from 5 to 2,500 pmol/L.

2. The method of claim 1, wherein the reaction solution (second PCR solution) comprises first PCR products obtained by polymerase chain reaction (first PCR) to amplify the first double-stranded DNA fragment, and the respective concentrations of primers remaining in the first PCR products

and primer dimers produced in the first PCR are less than 20 nmol/L in the second PCR solution.

3. The method of claim 2, wherein the respective concentrations of primers used for the first PCR are from 20 to 500 nmol/L.

4. The method of claim 2, wherein the second PCR is performed with the first PCR products which are diluted to 10- to 100-fold (at a final concentration in the second PCR solution).

5. The method of claim 2, further comprising a step of removing the primers and the primer dimers from the first PCR products.

6. The method of claim 2, wherein the first PCR is carried out using recombinant microorganisms or a culture broth thereof comprising the first double-stranded DNA fragment.

7. The method of any one of claims 1 to 6, wherein the second DNA fragment and/or the third DNA fragment is (are) single-stranded DNA(s) instead of double-stranded DNAs.

8. The method of any one of claims 1 to 7, wherein the sense primer and the anti-sense primer have the same nucleotide sequence.

9. The method of any one of claims 1 to 8, wherein the third DNA fragment comprises a transcription termination sequence.

10. The method of any one of claims 1 to 9, wherein at least one of the second DNA fragment and the third DNA fragment comprises a sequence coding for a tag peptide, and the tag peptide is synthesized by being fused with the protein or a portion thereof.

11. The method of claim 10, wherein the tag peptide is maltose binding protein, cellulose binding domain, glutathione-S-transferase, thioredoxin, streptavidin binding peptide or histidine tag peptide.

12. The method of claim 10, wherein the tag peptide is a histidine tag peptide consisting of the amino acid sequence of SEQ ID No. 1.

13. A method of producing a protein in a cell-free protein synthesis system using a template DNA that is produced by the method of any one of claims 1 to 12.